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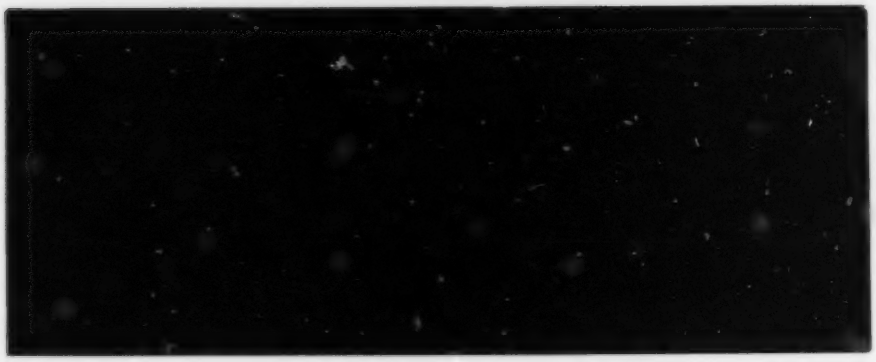

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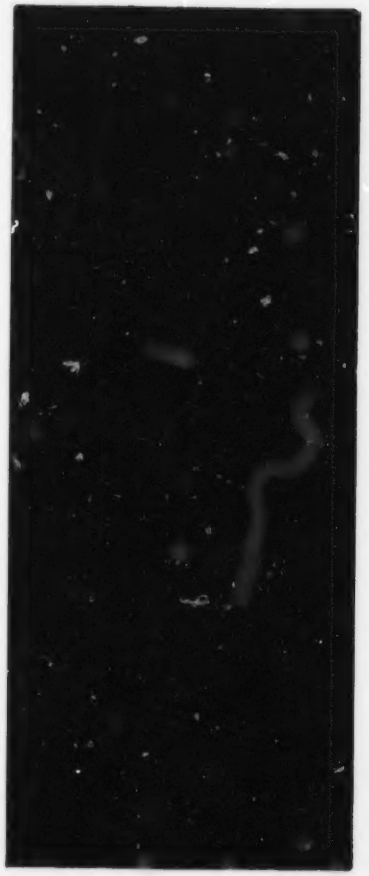

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Final Report

2.1-17

Embryonic Loss in Cattle Caused by Chromosomal Translocations

Conducted by
Dr. S. M. Schmutz and Dr. A. Barth

Funded by the Agriculture Development Fund

2.1-17

Final Report

Dr. S. M. Schmutz and Dr. A. Barth

February 22, 1990

Embryonic Loss in Cattle Caused by Chromosomal Translocations

Project No. 2.1-17

a) Executive Summary

Abstract

The current study was designed to measure the impact of the 1;29 translocation on bovine embryos when carried by either the dam or the sire. Six hundred and thirty-six ova and embryos were collected during this study. Fertilization appeared unaffected by this translocation. Translocation carrier cows did yield fewer embryos after superovulation than did cows with normal chromosomes.

Cattle carrying this translocation should theoretically produce gametes with four major chromosome arrangements. All of these were found amongst the 39 embryos karyotyped. Eight (20.5%) of these embryos would have died in utero because the chromosome arrangements were unbalanced. These lethal arrangements occurred more often when the cow carried the translocation than when the bull did.

Summary

The occurrence of a chromosome problem, a 1;29 translocation, due to the fusion of two chromosomes has been reported to reduce fertility in cattle. This translocation has been found to affect bulls of the continental beef breeds and British dairy breeds. Previous studies funded by the Agriculture Development Fund and Economic Regional Development Arrangement have shown that 8-10% Saskatchewan beef cattle do carry this translocation. The current study was designed to measure the impact of the 1;29 translocation on bovine embryos when carried by either the dam or the sire.

Embryos were collected from three mating groups: normal bull cross normal cow, normal bull cross translocation carrier cow, and translocation bull cross normal cow. The proportion of ova which were fertilized did not vary among the groups, indicating that fertilization rates were not affected by the translocation. The translocation cows did yield fewer embryos on average than did cows with normal karyotypes, which may suggest ovulation rates could be reduced (at least after superovulation attempts) as a result of the translocation.

Eight of 39 (20.5%) embryos karyotyped had abnormal karyotypes which would have resulted in embryonic loss. This is approximately twice as high as has been previously assessed based on cows open, or observed aborting. Since the numbers of embryos involved in this study are small, this loss in fertility should be viewed very cautiously. These embryos were collected at 7 days, which is prior to implantation. It is likely that some of these embryos may not have implanted and that the cow may have cycled back on schedule after the loss of such an early embryo. The true biological embryonic loss may indeed be quite high as a result of this translocation, but the practical loss to the producer is probably much less since the cow may recylce and be bred almost on schedule if natural service is in use.

If a translocation carrier is mated to an animal with normal chromosomes, two types of calves are expected to be produced with equal frequency. The liveborn calves could be either normal or translocation carriers. In addition, some embryos should be produced with one too many chromosomes (60,t) and some with one too few (59) but none should survive to birth. All four of these types of embryos were observed both when the dam was the carrier and when the bull was the carrier. In addition, some other unexpected embryos types were also found (61,XY and 58,XY,t). When

the cow was the carrier, 4 (36%) lethal karyotypes versus 7 viable karyotypes were observed compared to 5 (19%) lethal and 21 viable karyotypes when the bull was the carrier. This might suggest that the cow is more impaired in terms of her fertility as a result of this translocation compared to the bull as has been shown in similar human translocations. It must be emphasized that the numbers are small and this conclusion can not be made with certainty.

This study confirms that embryonic loss does play a major role in fertility reduction in both translocation carrier bulls and dams. Such early embryonic loss may be a significant cause of reduced fertility in some herds in Saskatchewan. Therefore proposed legislation and or recommendations that such translocation bulls be identified in A.I. centers, if not eliminated, seems warranted. A few progressive cattle breed associations are taking positive measures to educate their members about this problem and to suggest means of curtailing its spread.

b) **Technical Report**

(attached)

c) **Papers and Presentations**

Mapletoft, R. J., V. Pawlyshyn, A. Garcia, G. A. Bo, N. Wilmott, J. Saunders, and S. M. Schmutz. Comparison of four different gonadotropin treatments for inducing superovulation in cows with 1;29 translocation. Annual Embryo Transfer Meeting, Denver, Jan. 1990. (Poster presentation)

Schmutz, S. M. Informal discussion of results with the Board of Directors, Canadian Charolais Association, at their National Meeting on Feb. 8, 1990.

d) **Expenditure Statement**

(attached)

e) **Personnel**

Dr. Sheila Schmutz	20%
Dr. Albert Barth	5%
Dr. Reuben Mapletoft	5%
Mrs. Jane Moker	70%
Mrs. Marie Hanson, summer student	10%
Mr. Reynold Bergen, summer student	20%
Mr. Lester Price, summer student	20%
Mrs. Pam Bowman (semen preparation)	

f) **Equipment**

No equipment over $500 was purchased from these funds.

Final Technical Report

Dr. S. M. Schmutz and Dr. A. Barth

February 15, 1990

Embryonic Loss in Cattle Caused by Chromosomal Translocations

Project No. 2.1-17

Embryos were collected from three mating groups: normal bull cross normal cow, normal bull cross translocation carrier cow, and translocation bull cross normal cow. The proportion of ova which were fertilized did not vary among the groups, indicating that fertilization rates were not affected by the translocation. The translocation cows did yield fewer embryos on average than did cows with normal karyotypes, which may suggest ovulation rates could be reduced (at least after superovulation attempts) as a result of the translocation.

Eight of 39 (20.5%) embryos karyotyped had abnormal karyotypes which would have resulted in embryonic loss. These abnormal karyotypes represent all four of the theoretically predicted products of conception and two additional abnormal combinations. The proportion of embryos with such unbalanced karyotypes was slightly higher when the cow (36%) carried the translocation than when the bull (19%) did.

This study indicates that fertility is impaired due to the presence of this translocation. The major loss in reproductive fitness occurs due to embryonic loss rather than failure at fertilization.

Background

Previous studies have demonstrated that cattle with a translocation of chromosomes 1 and 29 have reduced fertility compared to cattle with normal karyotypes. The magnitude of this reduction and the reason, embryonic loss and/or failure to conceive, have not been determined however. This is important if one wishes to establish the cost-effectiveness of screening for such chromosomal abnormalities.

Refsdal (1976) has demonstrated that daughters of bulls with a 1/29 translocation had lower fertility compared to the Norwegian cattle population using a retrospective analysis of national records of non-return rates. Although a drop in fertility was demonstrated a more precise study needs to be done since data from all daughters, those with normal chromosomes and those with translocations, were pooled.

Dryendahl and Gustavsson (1979) found that there were significantly more females open at 28 and 56 days when a bull with a translocation was used compared to a bull with normal chromosomes. They were not able to determine whether the cows which were open at 28 days did so because of early embryonic loss or failure to conceive.

Few studies involving embryos from translocation 1;29 carriers have been done to date. King et al. (1980, 1981) successfully karyotyped 38 embryos sired by a bull carrying the 1/29 translocation and found 2 (5.3%) were abnormal. Popescu (1980) karyotyped 52 embryos sired by a carrier bull and again 2 (3.8%) were abnormal. King and Linares (1983) attempted to karyotype 3 embryos collected from one cow with the 1;29 translocation but were unsuccessful. Because these sample sizes were small and no female carriers had been studied, this study was conducted.

Objectives

1) To determine the degree to which fertility is reduced in cattle carrying translocations.

2) To elucidate whether reduced fertility is a result of embryonic loss or failure to conceive.

3) To compare the type and quantity of reproductive loss caused by translocations in males versus females.

4) To improve karyotyping methods in embryos for possible application to embryo sexing as a diagnostic service.

Experimental Methods

A Charolais bull has recently been found to carry a Robertsonian translocation of chromosomes 1 and 29. This bull was purchased by Dr. Albert Barth of the Western College of Veterinary Medicine and semen was collected for use in this study. Additional semen from a bull carrying a 1;29 translocation was donated by an A.I. center.

In order to maximize the number of embryos for study, cows were superovulated, most repeatedly. These cows were bred with semen from either translocation carrier or normal bulls.

Nine Charolais cows were obtained which carried the translocation. These cows were bred with semen from bulls with normal karyotypes. They were superovulated approximately every other cycle, some to a total of five times over 18 months.

Thirty-eight cows with normal karyotypes, most crossbred and a few purebred Charolais, were obtained. These cows were also superovulated, most only once and a few twice.

All cows were flushed to recover embryos at 7 days. The embryos were prepared for chromosomal analysis directly following the procedure first developed by Tarkowski (1966), modified in our lab. Small groups of animals, 4 to 12, were superovulated at any one time between June 1988 and September 1989. All cows were inseminated twice after showing standing heat, at twelve hour intervals.

Results

A total of 636 ova or embryos were collected from these cows. Of these 141 were not fertilized. (Several others were used in testing new unsuccessful fixation methods for chromosome preparation.) Table 1 presents the proportion of unfertilized ova in each of the three mating groups. The proportion unfertilized is not significantly different in the three groups (χ^2=3.1, P=0.21).

Table 1. Number of total ova and embryos collected and number unfertilized for each mating type.

Pair	Total	No. Unfertilized
Both normal	51	15 (29%)
Female t	197	34 (17%)
Male t	388	92 (24%)

The flushes from translocation cows yielded fewer embryos on average than did the flushes from cows with normal karyotypes. This may in part be due to increased fertility of crossbred cows. Most of the cows with normal karyotypes were crossbred cows. All of the cows with the translocation were purebred cows. However it may also mean that translocation cows do not respond to superovulation as well as normal cows. Although there was tremendous variability in response to superovulation between individuals and some intra-individual between flush variability, the ovulation data were found to be significantly different between translocation and normal cows ($z=-1.94$, $P=0.05$).

Table 2. Mean number of ova and embryos/flush for cows with the translocation compared to cows with a normal karyotype.

Karyotype	Mean	Range
Normal cows	7.4	0-46
Translocations cows	4.4	0-26

Chromosome analysis could only be performed on fertilized ova. Not all flushes resulted in fertilized embryos. Three hundred and seventy-four fertilized embryos were studied from 86 successful flushes. Complete chromosome analysis was possible with 39 embryos (10.4%) (Table 3). Eight of these 39 embryos (20.5%) would have died in utero because of

unbalanced karyotypes. The incidences of the balanced karyotypes, 60 and 59,t, deviated from the theoretical 1:1 ratio, χ^2=4.08, P=.04.

Partial analysis was possible in other embryos. In an additional 37 embryos, the translocation could be identified although the total number of chromosomes could not be counted. Therefore the translocation was observed in a total of 54 embryos of the 76 analyzed (71%). Both sex chromosomes could be positively identified in only 18 embryos, 11 female and 7 male. Although the X chromosome is usually evident, the Y chromosome can be difficult to distinguish from amongst the other small chromosomes.

Table 3. Number of embryos in each karyotypic category obtained for each mating type.

Pair	60	59,t	60,t	59	Other	Unknown
Both Normal	4	0	0	0	0	27
Female t	4	3	1	1	1[a]	112
Male t	11	9	1	2	2[b]	196

[a] 61,XY karyotype.
[b] 58,XY,t karyotype.

Discussion

It was disappointing that chromosome analysis could not be done on the majority of the embryos collected. The success rate for analysis was low for a number of reasons. All potentially fertilized embryos were included in the total number studied. Many of these would be discarded as poor quality, non-transferrable embryos by the industry. However on a few occassions, we were able to karyotype such embryos and therefore an attempt was made in each case. We believed it was important to include all fertilized embryos in this sample since it has been postulated that this translocation causes early embryonic loss. If all poor quality and/or

degenerating embryos were discarded, it is possible that the results could have been biased since these embryos may have been the ones with the abnormal karyotype. This is corroborated by a study done by Linares et al. (1980) in which they found that 17/20 (85%) of embryos from normal parents were alive at 7 days whereas only 14/44 (32%) of embryos with translocation carrier sires were alive.

In the previous study by King et al. (1980, 1981), such non-transferrable embryos may have been discarded if they followed the protocol they used in other studies. This may explain why they found only 2 of the 38 embryos they analyzed to have unbalanced (60,t) karyotypes, whereas we found 20% to have unbalanced karyotypes. Both of the abnormal embryos King et al. (1981) found were 60, t. Popescu (1980) found an even lower proportion of abnormal embryos 2/52 but he collected embryos13 days after ovulation and perhaps some of the abnormal embryos had already died by this time. Both of the abnormal embryos he found had only 59 chromosomes.

Failure to karyotype embryos resulted from two possible causes - no metaphases were detected or chromosomes were not sufficiently spread. Chromosomes can be analyzed in only a small portion of the total cell cycle, during cell division or mitosis at the stage of metaphase. Approximately 10-20 of all cells are in this stage at any given moment. Therefore since the embryos collected at 7 days in this study contained only a few cells (approximately 16-64), most cells were not in metaphase and some embryos had no metaphase cells. The major analytical problem was spreading of the chromosomes however. Several techniques to prevent clumping of chromosomes were tried but no method prevented this entirely.

We were unable to successfully sex a high proportion of the embryos, and therefore we do not feel that this method of sexing will solve the industrial demand for sexed embryos. It could not be used by industry even if the success rate were high, unless it could be adapted to split embryos, since this method can only be conducted on embryos which are killed in the process. Given the low overall success of karyotyping small and poor embryos during this study, a small piece of a transferrable embryo would probably yield insufficient material for successfull sexing in most cases.

Although the number of data obtained from karyotyped embryos are small, they are very significant. From previous studies, we know that only cattle with either 60 chromosomes (the normal karyotype) or 59,t (the balanced translocation karyotype) are viable. Therefore the eight embryos detected with either 60,t; 59; 61; or 58,t karyotypes would all have aborted. This proves that cattle carrying this translocation give rise

to embryos with abnormal karyotypes. Furthermore, the reduced fertility observed in such translocation carrier adults can be attributed in large part to embryonic loss. Unfortunately sperm and ova with abnormal chromosome numbers seem capable of participating in zygote formation (fertilization). The data on proportion of ova fertilized (Table 1) suggests that translocation carriers have no more difficulty conceiving than normal cattle. Translocation carrier cows did yield fewer embryos per flush on average than did normal cows (Table 2) and therefore female fertility may also be somewhat impaired at this level. Although translocation females seem to respond less well to superovulation, these data do not allow us to conclude that normal single ovulation would also occur at a reduced rate.

The two bulls with the translocation used in the course of this study both were capable of producing freezable semen of adequate count and morphology. Other studies (Dryendahl and Gustavsson 1979) found no evidence of any deficiency in sperm number or morphology as a result of this translocation.

Since 8 of 39 karyotyped embryos had abnormal chromosome complements, this could suggest that fertility should be reduced by up to 20.5%. This is approximately twice as high as has been previously assessed based on cows open, or observed aborting. Since the numbers of embryos involved in this study are small, this loss in fertility should be viewed very cautiously. These embryos were collected at 7 days, which is prior to implantation. It is likely that some of these embryos may not have implanted and that the cow may have cycled back on schedule after the loss of such an early embryo. The true biological embryonic loss may indeed be quite high as a result of this translocation, but the practical loss to the producer is probably much less since the cow may recylce and be bred almost on schedule if natural service is in use.

It should also be noted that all four expected products of conception were observed both when the dam was the carrier and when the bull was the carrier. When the cow was the carrier, 4 (36%) lethal karyotypes versus 7 viable karyotypes were observed compared to 5 (19%) lethal and 21 viable karyotypes when the bull was the carrier. This might suggest that the cow is more impaired in terms of her fertility as a result of this translocation compared to the bull as has been shown in similar human translocations. It must be emphasized that the numbers are small and this conclusion can not be made with certainty.

Conclusions and Impact

Simultaneous chromosomal studies of beef bulls in Saskatchewan indicate that the incidence of Robertsonian translocations may be relatively high, 8-10% in continental breeds. This study documents that embryonic loss does play a major role in fertility reduction in both translocation carrier bulls and dams. Such early embryonic loss may be a significant cause of reduced fertility in some herds in Saskatchewan. Therefore proposed legislation and or recommendations that such translocation bulls be identified in A.I. centers, if not eliminated, seems warranted.

Karyotyping of embryos for commercial embryo sexing does not appear promising. Results, although accurate, are costly and failure to obtain results may be high if only a small piece of the embryo were available. Therefore alternative methods, such as DNA sexing should be pursued to fill this commercial need.